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(71) Applicant *(for all designated States except US)*: **NEUTEC PHARMA PLC** [GB/GB]; St. James's Court, Brown Street, Manchester, Cheshire M2 2JF (GB).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **BURNIE, James, Peter** [GB/GB]; 1 Greystoke Drive, Alderley Edge, Cheshire SK9 7PY (GB). **MATTHEWS, Ruth, Christine** [GB/GB]; 1 Greystoke Drive, Alderley Edge, Cheshire SK9 7PY (GB).

(74) Agent: **MCNEIGHT & LAWRENCE**; Regent House, Heaton Lane, Stockport, Cheshire SK4 1BS (GB).

(81) Designated States *(national)*: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

**Published:**
— *With international search report.*

(88) Date of publication of the international search report:
7 December 2000

*For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.*

WO 00/46359 A3

(54) Title: MEDICAMENT

(57) Abstract: The present invention concerns treatment, prevention and diagnosis of infection due to *Chlamydia pneumoniae* and in particular to the prevention and treatment of atherosclerosis, including coronary atherosclerosis, caused by same.

# INTERNATIONAL SEARCH REPORT

Inter ial Application No
PCT/GB 00/00237

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N15/00 C07K14/295

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category ° | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X,P | WO 99 27105 A (GRIFFAIS REMY ;GENSET (FR))<br>3 June 1999 (1999-06-03)<br>abstract<br>page 1223 -page 1224<br>--- | 1-5,8-14 |
| X | EP 0 784 059 A (HITACHI CHEMICAL CO LTD)<br>16 July 1997 (1997-07-16)<br>the whole document<br>--- | 1-5,8-14 |
| X,P | KALMAN S. ET AL.: "Comparative genomes of Chlamydia pneumoniae and C. trachomatis"<br>NATURE GENETICS,<br>vol. 21, April 1999 (1999-04), pages 385-389, XP002141432<br>the whole document<br>---<br>-/-- | 1-3,11 |

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

| Date of the actual completion of the international search | Date of mailing of the international search report |
|---|---|
| 30 June 2000 | 11. 9. 00 |

| Name and mailing address of the ISA | Authorized officer |
|---|---|
| European Patent Office, P.B. 5818 Patentlaan 2<br>NL - 2280 HV Rijswijk<br>Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,<br>Fax: (+31-70) 340-3016 | Panzica, G |

Form PCT/ISA/210 (second sheet) (July 1992)

# INTERNATIONAL SEARCH REPORT

Inter al Application No
PCT/GB 00/00237

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

| Category ° | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| A | KORNAK JM ET AL: "Sequence analysis of the gene encoding the Chlamydia pneumoniae DnaK protein homolog" INFECTION AND IMMUNITY,US,AMERICAN SOCIETY FOR MICROBIOLOGY. WASHINGTON, vol. 59, no. 2, 1991, pages 721-725, XP002076846 ISSN: 0019-9567 the whole document --- | 1-5,8-14 |
| A | Y KANAMOTO ET AL: "Antigenic characterization of Chlamydia pneumoniae isolated in Hiroshima, Japan" MICROBIOLOGY AND IMMUNOLOGY,JP,TOKYO, vol. 37, no. 6, 1 January 1993 (1993-01-01), pages 495-498, XP002088968 ISSN: 0385-5600 the whole document --- | 1-5,8-14 |
| A | IIJIMA ET AL: "Characterization of Chlamydia pneumoniae species-specific proteins immunodominant in humans" JOURNAL OF CLINICAL MICROBIOLOGY,US,WASHINGTON, DC, vol. 32, no. 3, March 1994 (1994-03), pages 583-588-588, XP002115816 ISSN: 0095-1137 the whole document --- | 1-5,8-14 |
| A | PEREZ MELGOSA M ET AL: "Isolation and characterization of a gene encoding a Chlamydia pneumoniae 76-kilodalton protein containing a species-specific epitope" INFECTION AND IMMUNITY, vol. 62, no. 3, 1994, pages 880-886, XP002076845 ISSN: 0261-4189 the whole document ----- | 1-5,8-14 |

2

# INTERNATIONAL SEARCH REPORT

Ir. ational application No.
PCT/GB 00/00237

**Box I Observations where certain claims were found unsearchable (C ntinuation of item 1 of first sheet)**

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 12, 13 are directed to a diagnostic method and claim 14 to a method of treatment practised on the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [X] Claims Nos.: 6, 7
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

The subject-matter of claims 6 and 7 so lacks of clarity and so lacks of support in the present application that a search for said claims could not be performed, according to Articles 5 and 6 of PCT.

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

**Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)**

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [X] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-14 (in part)

**Remark on Protest**

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

1. Claims: 1-14 (in part)

Protein and nucleic acid from Chlamydia pneumoniae, as set forth respectively in SeqId.No.1 and 2 of the sequence listing, and uses of the same for methods of diagnosis and treatment.

2. Claims: 1-14 (in part)

Polypeptidic fragment from Chlamidia pneumoniae, having primary structure as set forth in Seq.Id.No.4 of the sequence listing, and uses of the same in methods of diagnosis and treatment.

3. Claims: 1-14 (in part)

Polypeptidic fragment from Chlamidia pneumoniae, having primary structure as set forth in Seq.Id.No.5 of the sequence listing, and uses of the same in methods of diagnosis and treatment.

4. Claims: 1-14 (in part)

Polypeptidic fragment from Chlamidia pneumoniae, having primary structure as set forth in Seq.Id.No.6 of the sequence listing, and uses of the same in methods of diagnosis and treatment.

5. Claims: 1-14 (in part)

Polypeptidic fragment from Chlamidia pneumoniae, having primary structure as set forth in Seq.Id.No.7 of the sequence listing, and uses of the same in methods of diagnosis and treatment.

6. Claims: 1-14 (in part)

Polypeptidic fragment from Chlamidia pneumoniae, having primary structure as set forth in Seq.Id.No.8 of the sequence listing, and uses of the same in methods of diagnosis and treatment.

7. Claims: 1-14 (in part)

Polypeptidic fragment from Chlamidia pneumoniae, having primary structure as set forth in Seq.Id.No.9 of the sequence listing, and uses of the same in methods of diagnosis and treatment.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

8. Claims: 1-14 (in part)

Polypeptidic fragment from Chlamidia pneumoniae, having primary structure as set forth in Seq.Id.No.10 of the sequence listing, and uses of the same in methods of diagnosis and treatment.

9. Claims: 1-14 (in part)

Polypeptidic fragment from Chlamidia pneumoniae, having primary structure as set forth in Seq.Id.No.11 of the sequence listing, and uses of the same in methods of diagnosis and treatment.

10. Claims: 1-14 (in part)

Polypeptidic fragment from Chlamidia pneumoniae, having primary structure as set forth in Seq.Id.No.12 of the sequence listing, and uses of the same in methods of diagnosis and treatment.

11. Claims: 1-14 (in part)

Polypeptidic fragment from Chlamidia pneumoniae, having primary structure as set forth in Seq.Id.No.13 of the sequence listing, and uses of the same in methods of diagnosis and treatment.

12. Claims: 1-14 (in part)

Polypeptidic fragment from Chlamidia pneumoniae, having primary structure as set forth in Seq.Id.No.14 of the sequence listing, and uses of the same in methods of diagnosis and treatment.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Claims Nos.: 6, 7

The subject-matter of claims 6 and 7 so lacks of clarity and so lacks of support in the present application that a search for said claims could not be performed, according to Articles 5 and 6 of PCT.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

# INTERNATIONAL SEARCH REPORT

Information on patent family members

Inten .al Apptication No
PCT/GB 00/00237

| Patent document cited in search report | Publication date | Patent family member(s) | Publication date |
|---|---|---|---|
| WO 9927105 A | 03-06-1999 | AU 1170299 A | 15-06-1999 |
| | | EP 1032674 A | 06-09-2000 |
| EP 0784059 A | 16-07-1997 | AU 685680 B | 22-01-1998 |
| | | AU 3532995 A | 09-04-1996 |
| | | WO 9609320 A | 28-03-1996 |
| | | JP 8143594 A | 04-06-1996 |
| | | JP 9009974 A | 14-01-1997 |
| | | JP 9009976 A | 14-01-1997 |
| | | JP 9009999 A | 14-01-1997 |
| | | JP 9015243 A | 17-01-1997 |
| | | JP 9015244 A | 17-01-1997 |

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

# PCT

## NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Assistant Commissioner for Patents
United States Patent and Trademark Office
Box PCT
Washington, D.C.20231
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

| | |
|---|---|
| **Date of mailing** (day/month/year)<br>04 October 2000 (04.10.00) | |
| **International application No.**<br>PCT/GB00/00237 | **Applicant's or agent's file reference**<br>M99/0035/PCT |
| **International filing date** (day/month/year)<br>28 January 2000 (28.01.00) | **Priority date** (day/month/year)<br>05 February 1999 (05.02.99) |
| **Applicant**<br>BURNIE, James, Peter et al | |

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

04 September 2000 (04.09.00)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

| | |
|---|---|
| **The International Bureau of WIPO**<br>**34, chemin des Colombettes**<br>**1211 Geneva 20, Switzerland** | Authorized officer<br>Pascal Piriou |
| Facsimile No.: (41-22) 740.14.35 | Telephone No.: (41-22) 338.83.38 |

GB0000237

Form PCT/IB/331 (July 1992)

PATENT COOPERATION TREATY

# PCT

## INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

| Applicant's or agent's file reference | FOR FURTHER ACTION | See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416) |
|---|---|---|
| M99/0035/PCT | | |
| International application No. | International filing date *(day/month/year)* | Priority date *(day/month/year)* |
| PCT/GB00/00237 | 28/01/2000 | 05/02/1999 |
| International Patent Classification (IPC) or national classification and IPC<br>C12N15/00 | | |
| Applicant<br>NEUTEC PHARMA PLC et al. | | |

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 8 sheets, including this cover sheet.

   ☐ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☒ Priority
   - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☒ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

| Date of submission of the demand | Date of completion of this report |
|---|---|
| 04/09/2000 | 21.05.2001 |
| Name and mailing address of the international preliminary examining authority:<br>European Patent Office<br>D-80298 Munich<br>Tel. +49 89 2399 - 0 Tx: 523656 epmu d<br>Fax: +49 89 2399 - 4465 | Authorized officer<br>Marinoni, J-C<br>Telephone No. +49 89 2399 8563 |

Form PCT/IPEA/409 (cover sheet) (January 1994)

# INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/GB00/00237

## I. Basis of th report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:
**Description, pages:**

1-21 as originally filed

**Claims, No.:**

1-14 as originally filed

**Sequence listing part of the description, pages:**

1-9, as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☒ contained in the international application in written form.
- ☒ filed together with the international application in computer readable form.
- ☐ furnished subsequently to this Authority in written form.
- ☐ furnished subsequently to this Authority in computer readable form.
- ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:
- ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

   *(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)*

6. Additional observations, if necessary:

**II. Priority**

1. ☐ This report has been established as if no priority had been claimed due to the failure to furnish within the prescribed time limit the requested:

   ☐ copy of the earlier application whose priority has been claimed.

   ☐ translation of the earlier application whose priority has been claimed.

2. ☐ This report has been established as if no priority had been claimed due to the fact that the priority claim has been found invalid.

   Thus for the purposes of this report, the international filing date indicated above is considered to be the relevant date.

3. Additional observations, if necessary:
   **see separate sheet**

**III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability**

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

   ☐ the entire international application.

   ☒ claims Nos. 6, 7, 9 completely; 10, 11 and 14 partially.

because:

   ☐ the said international application, or the said claims Nos. relate to the following subject matter which does not require an international preliminary examination (*specify*):

   ☒ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. 9 completely; 10, 11 and 14 all partially are so unclear that no meaningful opinion could be formed (*specify*):
   **see separate sheet**

   ☒ the claims, or said claims Nos. 9 completely; 10, 11 and 14 all partially are so inadequately supported by the description that no meaningful opinion could be formed.

   ☒ no international search report has been established for the said claims Nos. 6 and 7 both completely.

2. A meaningful international preliminary examination cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

   ☐ the written form has not been furnished or does not comply with the standard.

   ☐ the computer readable form has not been furnished or does not comply with the standard.

**IV. Lack of unity of invention**

1. In response to the invitation to restrict or pay additional fees the applicant has:

   ☐ restricted the claims.

   ☐ paid additional fees.

   ☐ paid additional fees under protest.

   ☐ neither restricted nor paid additional fees.

2. ☐ This Authority found that the requirement of unity of invention is not complied and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rules 13.1, 13.2 and 13.3 is

   ☐ complied with.

   ☒ not complied with for the following reasons:
   **see separate sheet**

4. Consequently, the following parts of the international application were the subject of international preliminary examination in establishing this report:

   ☐ all parts.

   ☒ the parts relating to claims Nos. 1-5, 8, 10-14 all partially.

**V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

1. Statement

| | | | |
|---|---|---|---|
| Novelty (N) | Yes: | Claims | 1-5, 8, 10-14 |
| | No: | Claims | none |
| Inventive step (IS) | Yes: | Claims | none |
| | No: | Claims | 1-5, 8, 10-14 |
| Industrial applicability (IA) | Yes: | Claims | 1-5, 8, 10-14 |
| | No: | Claims | none |

2. Citations and explanations
   **see separate sheet**

**VIII. Certain observations on the international application**

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
**see separate sheet**

**Re Item II**

**Priority**

The document KALMAN et al. 'Comparative genomes of *Chlamydia pneumoniae* and *C. trachomatis*' NATURE GENET., Vol. 21, April 1999, pages 385-389, was cited as a P-document.
However, the claimed priority date is considered to be valid for the subject-matter of the invention to which the following opinion applies (see **item III**). The document is therefore not taken into account for the establishement of the following report.

**Re Item III**

**Non-establishment of opinion with regard to novelty, inventive step and industrial applicability**

The subject-matter of **claims 6 and 7** was not searched (see the International Search Report). Therefore, no examination can be carried out.

Consequently, no opinion can be formulated on the subject-matter of **claim 9** (completely) which refers specifically to claims 6 and 7, but also **claims 10, 11 and 14** partially (binding agents and inhibitors).

**Re Item IV**

**Lack of unity of invention**

The International Search Authority raised an objection for lack of unity under Rule 13 PCT and subsequently identified 12 inventions. In the absence of payment of an additional search fee, the search has been limited to identified invention 1 (protein and nucleic acid from *Chlamydia pneumoniae*, as set forth respectively in SEQ ID No.1 and 2 of the sequence listing, and uses of the same for methods of diagnosis and treatment).
The following report is therefore restricted to invention 1 (**claims 1-5, 8 and 10-14** all partially).

**Re Item V**

**Reasoned statement under Article 35(2) with regard to novelty, inventiv step or industrial applicability; citations and explanations supporting such statement**

Reference is made to the following document:
**D1**: EP 0 784 059, 16 July 1997.

1. **Claim 1** is directed to a protein having the amino acid sequence of SEQ ID No. 2. Document **D1** discloses an antigenic polypeptide of *C. pneumoniae* having the sequence shown in SEQ ID No.1 (or SEQ ID No. 15, from aa 162 to aa 469; see page 22, lines 1-5) and corresponding to aa 3 to 490 of SEQ ID No. 2 of the present application.
   Therefore, the protein of SEQ ID No.2 is novel.

2. However, in view of **D1**, which is considered to represent the closest prior art, the technical problem underlying the present application appears to reside in the cloning of a longer (full-length?) *C. pneumoniae* protein, parts of which (among them the part disclosed in **D1**) are already known to be recognized by antisera from patients (*i.e.* antigenic) and therefore be used as diagnostics or to (possibly) elicit an immune response (thereby having a potential therapeutic utility), see **D1**, page 22, lines 30-41; page 25, lines 8-10; page 25, lines 45-47. The provision of proteins including the polypeptide of SEQ ID No.1 of **D1** is known from **D1** (see claims 4 and 5).
   In the present application, the protein of SEQ ID No.2 is merely a variant (at best, the full-length protein) of the protein of **D1** for which no inventive step can be acknowledged for the reason that (i) this protein has no demonstrated function, (ii) **D1** suggests to construct polypeptides containing said sequence, and that (iii) the mere use as an antigen and/or as a medicament/diagnostic tool of this protein or variants thereof is already foreseen in the prior art.

3. Consequently, the subject-matter of **claims 1-5, 8, and 10-14** (all partially) fail to meet the requirements of Article 33(3) PCT concerning inventive step.

**Re Item VI**
**Certain documents cited**

Certain published documents (Rule 70.10)

| Application No Patent No | Publication date *(day/month/year)* | Filing date *(day/month/year)* | Priority date *(valid claim)* *(day/month/year)* |
|---|---|---|---|

| | | | |
|---|---|---|---|
| WO 99/27105 | 03/06/1999 | 20/11/1998 | 21/11/1997 |

**Re Item VIII**

**Certain observations on the international application**

1. In the present application, the assumption that the claim polypeptide could be used as a medicament relies only in its ability to be recognized by a human antibody from a antibody library originating from a patient infected with *C. pneumoniae*. None of the example actually demonstrate that the claim polypeptide could elicit an immune response. The same is true for the polypeptides of **D1**. Therefore, it is considered that the polypeptide claimed in the present application has no additional properties compared to the polypeptides of **D1**.

2. The protein of **claim 1** is partially defined by its use ("for use in a method of treatment or diagnosis of the human or animal body). The mere expression of the intended use of said protein does not render said protein novel. Furthermore, clarity objections under Article 6 PCT may be raised since it is not clear whether the use of the protein is tentatively claimed (see the Guidelines, Ch. III, 4.8a).

PATENT COOPERATION TREATY

# PCT

## INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

| Applicant's or agent's file reference | FOR FURTHER ACTION see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below. | |
|---|---|---|
| M99/0035/PCT | | |
| International application No. | International filing date *(day/month/year)* | (Earliest) Priority Date *(day/month/year)* |
| PCT/GB 00/00237 | 28/01/2000 | 05/02/1999 |
| Applicant | | |
| NEUTEC PHARMA PLC et al. | | |

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 8 sheets.

☐ It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

a. With regard to the **language**, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

☐ the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

☒ contained in the international application in written form.

☒ filed together with the international application in computer readable form.

☐ furnished subsequently to this Authority in written form.

☐ furnished subsequently to this Authority in computer readble form.

☐ the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

☐ the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. ☒ **Certain claims were found unsearchable** (See Box I).

3. ☒ **Unity of invention is lacking** (see Box II).

4. With regard to the **title,**

☒ the text is approved as submitted by the applicant.

☐ the text has been established by this Authority to read as follows:

5. With regard to the **abstract,**

☒ the text is approved as submitted by the applicant.

☐ the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No. _____

☐ as suggested by the applicant.

☐ because the applicant failed to suggest a figure.

☐ because this figure better characterizes the invention.

☐ None of the figures.

# INTERNATIONAL SEARCH REPORT

Interna[redacted]al Application No
PC[redacted] 00/00237

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N15/00 C07K14/295

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category ° | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X,P | WO 99 27105 A (GRIFFAIS REMY ;GENSET (FR))<br>3 June 1999 (1999-06-03)<br>abstract<br>page 1223 -page 1224 | 1-5,8-14 |
| X | EP 0 784 059 A (HITACHI CHEMICAL CO LTD)<br>16 July 1997 (1997-07-16)<br>the whole document | 1-5,8-14 |
| X,P | KALMAN S. ET AL.: "Comparative genomes of Chlamydia pneumoniae and C. trachomatis"<br>NATURE GENETICS,<br>vol. 21, April 1999 (1999-04), pages 385-389, XP002141432<br>the whole document | 1-3,11 |

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

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"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

| Date of the actual completion of the international search | Date of mailing of the international search report |
|---|---|
| 30 June 2000 | 11. 9. 00 |

| Name and mailing address of the ISA | Authorized officer |
|---|---|
| European Patent Office, P.B. 5818 Patentlaan 2<br>NL - 2280 HV Rijswijk<br>Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,<br>Fax: (+31-70) 340-3016 | Panzica, G |

2

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

| Category ° | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| A | KORNAK JM ET AL: "Sequence analysis of the gene encoding the Chlamydia pneumoniae DnaK protein homolog" INFECTION AND IMMUNITY,US,AMERICAN SOCIETY FOR MICROBIOLOGY. WASHINGTON, vol. 59, no. 2, 1991, pages 721-725, XP002076846 ISSN: 0019-9567 the whole document --- | 1-5,8-14 |
| A | Y KANAMOTO ET AL: "Antigenic characterization of Chlamydia pneumoniae isolated in Hiroshima, Japan" MICROBIOLOGY AND IMMUNOLOGY,JP,TOKYO, vol. 37, no. 6, 1 January 1993 (1993-01-01), pages 495-498, XP002088968 ISSN: 0385-5600 the whole document --- | 1-5,8-14 |
| A | IIJIMA ET AL: "Characterization of Chlamydia pneumoniae species-specific proteins immunodominant in humans" JOURNAL OF CLINICAL MICROBIOLOGY,US,WASHINGTON, DC, vol. 32, no. 3, March 1994 (1994-03), pages 583-588-588, XP002115816 ISSN: 0095-1137 the whole document --- | 1-5,8-14 |
| A | PEREZ MELGOSA M ET AL: "Isolation and characterization of a gene encoding a Chlamydia pneumoniae 76-kilodalton protein containing a species-specific epitope" INFECTION AND IMMUNITY, vol. 62, no. 3, 1994, pages 880-886, XP002076845 ISSN: 0261-4189 the whole document ----- | 1-5,8-14 |

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# INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/GB 00/00237

| Patent document cited in search report | Publication date | Patent family member(s) | Publication date |
|---|---|---|---|
| WO 9927105 A | 03-06-1999 | AU 1170299 A | 15-06-1999 |
| | | EP 1032674 A | 06-09-2000 |
| EP 0784059 A | 16-07-1997 | AU 685680 B | 22-01-1998 |
| | | AU 3532995 A | 09-04-1996 |
| | | WO 9609320 A | 28-03-1996 |
| | | JP 8143594 A | 04-06-1996 |
| | | JP 9009974 A | 14-01-1997 |
| | | JP 9009976 A | 14-01-1997 |
| | | JP 9009999 A | 14-01-1997 |
| | | JP 9015243 A | 17-01-1997 |
| | | JP 9015244 A | 17-01-1997 |

# INTERNATIONAL SEARCH REPORT

[redacted]tional application No.
PCT/GB 00/00237

**Box I Observations wh re certain laims w re f und unsearchabl (C ntinuation of it m 1 of first sh t)**

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 12, 13 are directed to a diagnostic method and claim 14 to a method of treatment practised on the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [X] Claims Nos.: 6, 7
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

The subject-matter of claims 6 and 7 so lacks of clarity and so lacks of support in the present application that a search for said claims could not be performed, according to Articles 5 and 6 of PCT.

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

**Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)**

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [X] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-14 (in part)

**Remark on Protest**

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

1. Claims: 1-14 (in part)

Protein and nucleic acid from Chlamydia pneumoniae, as set forth respectively in SeqId.No.1 and 2 of the sequence listing, and uses of the same for methods of diagnosis and treatment.

2. Claims: 1-14 (in part)

Polypeptidic fragment from Chlamidia pneumoniae, having primary structure as set forth in Seq.Id.No.4 of the sequence listing, and uses of the same in methods of diagnosis and treatment.

3. Claims: 1-14 (in part)

Polypeptidic fragment from Chlamidia pneumoniae, having primary structure as set forth in Seq.Id.No.5 of the sequence listing, and uses of the same in methods of diagnosis and treatment.

4. Claims: 1-14 (in part)

Polypeptidic fragment from Chlamidia pneumoniae, having primary structure as set forth in Seq.Id.No.6 of the sequence listing, and uses of the same in methods of diagnosis and treatment.

5. Claims: 1-14 (in part)

Polypeptidic fragment from Chlamidia pneumoniae, having primary structure as set forth in Seq.Id.No.7 of the sequence listing, and uses of the same in methods of diagnosis and treatment.

6. Claims: 1-14 (in part)

Polypeptidic fragment from Chlamidia pneumoniae, having primary structure as set forth in Seq.Id.No.8 of the sequence listing, and uses of the same in methods of diagnosis and treatment.

7. Claims: 1-14 (in part)

Polypeptidic fragment from Chlamidia pneumoniae, having primary structure as set forth in Seq.Id.No.9 of the sequence listing, and uses of the same in methods of diagnosis and treatment.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

8. Claims: 1-14 (in part)

Polypeptidic fragment from Chlamidia pneumoniae, having primary structure as set forth in Seq.Id.No.10 of the sequence listing, and uses of the same in methods of diagnosis and treatment.

9. Claims: 1-14 (in part)

Polypeptidic fragment from Chlamidia pneumoniae, having primary structure as set forth in Seq.Id.No.11 of the sequence listing, and uses of the same in methods of diagnosis and treatment.

10. Claims: 1-14 (in part)

Polypeptidic fragment from Chlamidia pneumoniae, having primary structure as set forth in Seq.Id.No.12 of the sequence listing, and uses of the same in methods of diagnosis and treatment.

11. Claims: 1-14 (in part)

Polypeptidic fragment from Chlamidia pneumoniae, having primary structure as set forth in Seq.Id.No.13 of the sequence listing, and uses of the same in methods of diagnosis and treatment.

12. Claims: 1-14 (in part)

Polypeptidic fragment from Chlamidia pneumoniae, having primary structure as set forth in Seq.Id.No.14 of the sequence listing, and uses of the same in methods of diagnosis and treatment.

**FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210**

Continuation of Box I.2

Claims Nos.: 6, 7

The subject-matter of claims 6 and 7 so lacks of clarity and so lacks of support in the present application that a search for said claims could not be performed, according to Articles 5 and 6 of PCT.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

# PCT

## NOTIFICATION CONCERNING SUBMISSION OR TRANSMITTAL OF PRIORITY DOCUMENT

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| Date of mailing (day/month/year)<br>13 March 2000 (13.03.00) | |
| Applicant's or agent's file reference<br>M99/0035/PCT | **IMPORTANT NOTIFICATION** |
| International application No.<br>PCT/GB00/00237 | International filing date (day/month/year)<br>28 January 2000 (28.01.00) |
| International publication date (day/month/year)<br>Not yet published | Priority date (day/month/year)<br>05 February 1999 (05.02.99) |
| Applicant<br>NEUTEC PHARMA PLC et al | |

1. The applicant is hereby notified of the date of receipt (except where the letters "NR" appear in the right-hand column) by the International Bureau of the priority document(s) relating to the earlier application(s) indicated below. Unless otherwise indicated by an asterisk appearing next to a date of receipt, or by the letters "NR", in the right-hand column, the priority document concerned was submitted or transmitted to the International Bureau in compliance with Rule 17.1(a) or (b).

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3. An **asterisk(*)** appearing next to a date of receipt, in the right-hand column, denotes a priority document submitted or transmitted to the International Bureau but not in compliance with Rule 17.1(a) or (b). In such a case, **the attention of the applicant is directed** to Rule 17.1(c) which provides that no designated Office may disregard the priority claim concerned before giving the applicant an opportunity, upon entry into the national phase, to furnish the priority document within a time limit which is reasonable under the circumstances.

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| 05 Febr 1999 (05.02.99) | 9902555.3 | GB | 06 Marc 2000 (06.03.00) |

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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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A2

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(30) Priority Data:
9902555.3 5 February 1999 (05.02.99) GB

(71) Applicant *(for all designated States except US)*: NEUTEC PHARMA PLC [GB/GB]; St. James's Court, Brown Street, Manchester, Cheshire M2 2JF (GB).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: BURNIE, James, Peter [GB/GB]; 1 Greystoke Drive, Alderley Edge, Cheshire SK9 7PY (GB). MATTHEWS, Ruth, Christine [GB/GB]; 1 Greystoke Drive, Alderley Edge, Cheshire SK9 7PY (GB).

(74) Agent: MCNEIGHT & LAWRENCE; Regent House, Heaton Lane, Stockport, Cheshire SK4 1BS (GB).

(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

**Published**
*Without international search report and to be republished upon receipt of that report.*

(54) Title: MEDICAMENT

(57) Abstract

The present invention concerns treatment, prevention and diagnosis of infection due to *Chlamydia pneumoniae* and in particular to the prevention and treatment of atherosclerosis, including coronary atherosclerosis, caused by same.

*FOR THE PURPOSES OF INFORMATION ONLY*

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# Medicament

The present invention concerns treatment, prevention and diagnosis of infection due to *Chlamydia pneumoniae* and in particular to the prevention and treatment of atherosclerosis, including coronary atherosclerosis, caused by same.

*C. pneumoniae* is associated with atherosclerosis but no definitive link between the two has yet been established (Hammerschlag, M.R., 1998, Eur. J. Clin. Microbiol. Infect. Dis., 17: 305-308). Friedank, H.M. *et al.* (1993, Eur. J. Clin. Microbiol. Infect. Dis., 12(12): 947-951) identify a 54 kDa *C. pneumoniae* antigen which was recognised by 93% of sera positive for *C. pneumoniae*, the antigen appearing to be located on the surface of elementary bodies. Wiedman, A.A.M. *et al.* (1997, Clin. Diagn. Labs. Immunol., 4(6):700-704) showed the infectivity of *C. pneumoniae* elementary bodies to be slightly reduced by the use of antibody specific against a 54 kDa *C. pneumoniae* protein.

Despite investigating it, other researchers have not confirmed the immunogenicity of the *C. pneumoniae* 54 kDa band (see for example Kutlin, A. and Roblin, P.M., 1998, J. Infect. Dis., 177: 720-724; Campbell, L.A. *et al.*, 1990, J. Clin. Microbiol., 28(6): 1261-1264; Campbell, L.A. *et al.*, 1990. Infection and Immunity, 58(1): 93-97; Puolakkainen, M. *et al.*, 1993, J. Clin. Microbiol., 31(8): 2212-2214; hkima, Y. *et al.*, 1994, J. Clin. Microbiol., 32(3): 583-588; Maass, M. and Gieffers, J., 1997, J. Infection, 35: 171-176; Gonen, R. *et al.*, 1993, APMIS, 101:719-726).

The present inventor has now succeeded in isolating, purifying and identifying a *C. pneumoniae* protein which (together with inhibitors of same, such as

antibodies) is protective and therapeutic against *C. pneumoniae* infection. The therapeutic role of the protein has previously neither been suggested nor disclosed.

According to the present invention there is provided a *C. pneumoniae* protein having the amino acid sequence of SEQ ID NO: 2, for use in a method of treatment or diagnosis of the human or animal body. The amino acid sequence has been confirmed by N-terminal amino-acid sequencing (see "Experimental" below) and the protein has a theoretical molecular weight of 50.8 kDa, although post-translational modifications such as glycosylation may of course affect its apparent molecular weight as determined by e.g. SDS-PAGE. Experiments (below) have shown it to have an apparent molecular weight of 51 kDa on SDS-PAGE gels.

As can be seen from the plethora of publications above, although some identify immunogenic bands at molecular weights of 50-54 kDa, no specific therapeutically effective proteins have been identified.

Experiments (below) have allowed the present inventor to isolate and purify the protein of the present invention and identify the gene sequence coding for the protein. This has allowed the determination of the protein amino acid sequence (above). The nucleotide sequence coding for same forms another part of the present invention. Thus according to the present invention there is also provided a nucleotide sequence coding for a protein according to the present invention, for use in a method of treatment or diagnosis of the human or animal body. Such a nucleotide sequence may have the sequence of SEQ ID NO: 1. Modified nucleotide sequences having codons encoding the same amino acid sequence will be readily apparent to one skilled in the art.

The nucleotide sequence of the present invention and the amino acid sequence it encodes are already known from the Chlamydia Genome Project

(*C. pneumoniae* CWL029/CPn0809), as is an apparent *C. trachomatis* homologue (CT578). However, therapeutic and diagnostic uses for same have not been previously suggested.

The invention also extends to encompass forms of the protein which have been insubstantially modified (i.e. which have been partially modified), particularly forms of the protein which display the same immunogenic properties as the protein itself.

By "partial modification" and "partially modified" is meant, with reference to amino acid sequences, a partially modified form of the molecule which retains substantially the properties of the molecule from which it is derived, although it may of course have additional functionality. Partial modification may, for example, be by way of addition, deletion or substitution of amino acid residues. Substitutions may be conserved substitutions. Hence the partially modified molecule may be a homologue of the molecules from which it was derived. It may, for example, have at least 70% homology with the molecule from which it was derived. It may for example have at least 80, 90 or 95% homology with the molecule from which it was derived. An example of a homologue is an allelic mutant.

Also provided according to the present invention is the use of a protein, immunogenic fragment thereof or nucleic acid sequence encoding same according to the present invention in the manufacture of a medicament for the treatment of infection due to *C. pneumoniae*.

Immunogenic fragments of the protein include any fragment of the protein which elicits an immune response, and includes epitopes. Analogues (mimotopes) of epitopes may be readily created, the mimotopes having different sequences but displaying the same epitope and thus the term "immunogenic fragments" also

encompasses immunogenic analogues of the fragments e.g. mimotopes. Epitopes may be readily determined and mimotopes readily designed (Geysen, H.M. *et al.*, 1987, Journal of Immunological Methods, 102: 259-274; Geysen, H.M. *et al.*,1988, J. Mol. Recognit., 1(1):32-41; Jung, G. and Beck-Sickinger, A.G., 1992, Angew. Chem. Int. Ed. Eng., 31: 367-486). Such an immunogenic fragment carrying epitopes may also be described as being a peptide having the amino acid sequence of the immunogenic fragment and which carries an epitope.

The present inventor has succeeded in isolating a number of epitopes (immunogenic fragments) of the protein of the present invention. Thus according to the present invention there is also provided an epitope having the amino acid sequence of any one of SEQ ID NOs: 4-14. In particular, SEQ ID NOs: 5-7 provide an overlapping set of highly immunogenic peptides - as can be seen from the experimental data (below) SEQ ID NO: 5 provides for especially good results. Similarly, excellent results are also obtained from SEQ ID NO: 8.

The protein, immunogenic fragments thereof and nucleic acid sequences encoding same may be used in therapy, both prophylactically (e.g. as immunostimulants such as vaccines) and for treatment of infection due to *C. pneumoniae*. For example a nucleotide sequence encoding the protein or immunogenic fragment thereof may be used in the manufacture of a DNA vaccine (Montgomery, D.L. *et al.*, 1997, Pharmacol. Ther., 74(2): 195-205; Donnelly, J.J. *et al.*, 1997, Annu. Rev. Immunol., 15: 617-648; Manickan, E. *et al.*, 1997, Crit. Rev. Immunol., 17(2): 139-154).

Binding agents and inhibitors (such as antibodies or other neutralising agents) specific against the protein and immunogenic fragments thereof may also be used both diagnostically and therapeutically. Binding agents have a target to which they are specific, and in the case of a binding agent being an antibody, the target is an antigen.

An example of a therapeutic medicament is antibody specific against the protein of the present invention, and this may be employed in immunotherapy, for example passive immunotherapy. Antibodies, their manufacture and use are well known (Harlow, E. and Lane, D., "Using Antibodies - A Laboratory Manual", Cold Spring Harbor Laboratory Press, New York, 1998) and so antibodies and antigen binding fragments thereof will be readily apparent to one skilled in the art, and reference herein to antibodies is also reference to antigen binding fragments unless stated otherwise. Other inhibitors such as ribozymes, antisense oligonucleotides and DNA vaccines will be readily apparent to one skilled in the art (Fries, P.C., 1999, "DNA Vaccines", New England Journal of medicine, 341: 1623-1624; Leitner, W.W. *et al.*, 1999, "DNA and RNA based vaccines: principles, progress and prospects", Vaccine, 18: 765-777; Muotri, A.R. et al., 1999, "Ribozymes and the anti-gene therapy: how a catalytic RNA can be used to inhibit gene function", Gene, 237: 303-310; Rossi, J.J., 1999, "Ribozymes, genomics and therapeutics", Chemistry & Biology, 6: R33-R37; James, H.A., 1999, "The potential application of ribozymes for the treatment of haematological disorders", Journal of Leukocyte Biolofy, 66: 361-368)

Thus the present invention also provides the use of a inhibitor specific to the protein of the present invention in the manufacture of a medicament for the treatment of infection due to *C.pneumoniae.*

Also provided according to the present invention is a method of manufacture of a medicament for the treatment of infection due to *C. pneumoniae*, characterised in the use of a protein, immunogenic fragment or inhibitor according to the present invention.

Also provided according to the present invention is a method of treatment of infection due to *C. pneumoniae*(e.g. of a patient in need of same), comprising the step

of administering to a patient a medicament comprising a protein, immunogenic fragment or inhibitor according to the present invention. The exact dose of medicament administered to a patient may be readily determined using simple dose-response assays. Medicaments may additionally comprise a pharmaceutically acceptable carrier, diluent or excipient (Remington's Pharmaceutical Sciences and US Pharmacopeia, 1984, Mack Publishing Company, Easton, PA, USA)

It has not been previously suggested that the protein of the present invention (or immunogenic fragments of same) is diagnostic for infection due to *C. pneumonia*. Binding agents specific to the protein of the present invention (for example antibodies) may also be used diagnostically, for example in an ELISA-type test. Thus also provided according to the present invention is the use of a protein, immunogenic fragment or binding agent according to the present invention in the manufacture of a diagnostic test for *C. pneumoniae*.

Also provided is a diagnostic test method for infection due to *C. pneumoniae* comprising the steps of:

I) reacting an antibody specific against the protein of the present invention with serum from a patient;

ii) detecting an antibody-antigen binding reaction; and

iii) correlating the detection of an antibody-antigen binding reaction with the presence of the protein.

Such test methods may also be performed using other binding agents specific to the protein of the present invention.

Also provided is a kit of parts for performing such a test, characterised in that it comprises antibody specific against the protein of the present invention.

The invention will be further apparent from the following description, with reference to the several figures of the accompanying drawings, which show, by way of example only, uses of the proteins of the present invention.

## EXPERIMENTAL

The experiments below detail the identification of a number of peptides and antisera against same which are useful in the therapy and diagnosis of infections due to *Chlamydia pneumoniae*. Starting with sera from infected patients, blotting against clinical isolates of *Chlamydia pneumoniae* showed the presence of an immunodominant antigen with an apparent molecular weight of 51 kDa, the antigen being stable to and released by octylglucoside treatment. N-terminal amino acid sequencing of the protein of the 51 kDa band allowed sequence database probing, in turn identifying a *C. pneumoniae* protein and a *C. trachomatis* homologue. Epitope mapping allowed the identification of antigenic peptides, which together with antibody against them were tested for their therapeutic and diagnostic efficacy.

Western Blotting - Using the Novex nuPAGE Electrophoresis System.

1. SDS PAGE

*Preparation of Sample:*

1. 100 µl of Novex SDS Sample loading buffer was added to 400 µl of a preparation of a *Chlamydia pneumoniae* clinical isolate and the mixture placed into a boiling waterbath for 10 minutes.

2. 10 µl of the mixture was loaded into each well of a Novex 4-12% Bis-Tris NuPage gel (1.0 mm, 12 well). In addition, 4 µl of Novex Multimark molecular weight standards were added to a single well on each gel.

3. Electrophoresis was performed using 1x Novex MOPS electrophoresis buffer at 200v for 40 minutes.

*Western Transfer Protocol:*

1. The blotting apparatus and the gel membrane "sandwiches" were assembled according to the protocol described in the Novex instruction booklet provided with the gels.

2. Blotting was performed using 1x Novex Transfer buffer containing 20% methanol. Transfer was carried out at 30v (constant) for 1 hour.

3. Following transfer, the membranes were removed from the apparatus and left to "Block" overnight in 3% Bovine Serum Albumin (BSA) at 4 °C.

*Probing With Patient's Serum:*

1. The membranes were cut into strips and placed into the wells of incubation trays. Patients' serum was diluted 1 in 20 in 3% BSA and 2 ml added to each strip. (2 strips per patient).

2. The membranes were incubated at room temperature for 2 hours with agitation.

3. The strips were washed 5 times over 30 minutes with 0.85% NaCl/0.01% Tween 20.

4. 2 ml of goat anti-human IgM or IgG alkaline phosphatase conjugated anti-immunoglobulin diluted 1 in 4000 in 3% BSA were added to each strip. The strips were incubated for a further hour at room temperature with agitation.

5. The membranes were washed a further 5 times as previously described.

6. Antibody-antigen interaction was visualised by the addition of NBT/BCIP (50 mg/ml) in pH 9.5 phosphate buffer.

7. The reaction was allowed to proceed until the bands had reached the required intensity.

**Sera**

Group A: Children with respiratory tract infection and no evidence of *Chlamydia pneumoniae* as shown by negative microimmunofluorescence (less than 1 in 64) test (n=19).

Group B: Children with respiratory tract infection and a microimmunofluorescence titre greater than 1 in 512 (n=18).

Group C: Patients undergoing cardiac surgery for advanced coronary disease (n=32). Ten of these had antibody on immunoblot.

Group D: Adults with respiratory tract infection and a chlamydia complement fixation test greater than 1 in 40 (n=27) using LGV 2 as an antigen.

Group E: Adults with pelvic inflammatory disease due to *Chlamydia trachomatis* (n=21).

Group F: Sera (n=11) which were positive for the 60/62 kDa doublet and band at 51 kDa were retested on antigen prepared from *Chlamydia pneumoniae* where the purified elementary bodies were incubated with 1% octylglucoside at 37 °C for 30 minutes rather than in SDS.

Results:

Results of the sera blotting experiments are shown in Table 1. It should be noted that sera blotting determines the presence in patients of antibodies specific against a given antigen, and so when a patient has previously been infected by a pathogen and developed an immune response against an antigen, that immune response may still be detectable at a later date when the patient is no longer infected. Hence background results must be interpreted in light of the general infection of a population by the pathogen. For example, the general population has an infection rate by adulthood of approximately 10% for *C. pneumoniae*, thus a background rate of detection of *C. pneumoniae* antigens of up to 10% should be expected.

**Conclusions:**

The sera from Group A children did not recognise *C.pneumoniae* on immunoblot. The Group B sera from children with evidence of *C.pneumoniae* infection recognised a range of antigens with apparent molecular weights ranging from 30 to 180 kDa. IgM for an antigen complex at 60/62 kDa which occurred as a doublet was immunodominant as well as an antigen at 51 kDa. For IgG the antibody was most pronounced for the antigen at 51 kDa. In the cardiac patients, 23 produced antibody and this was for IgM against the bands at 67, 60/62 and 51 kDa. For IgG this was the band at 51 kDa. For Group D IgM was most pronounced for the 60/62 kDa doublet and IgG for the band at 180 kDa and the doublet at 60/62 kDa. This group of sera contains those with infection most likely due to *Chlamydia psittaci*. The sera from Group E patients infected with *Chlamydia trachomatis* did not cross-react.

**Group F Sera**

On re-blotting with those sera previously positive for the 60/62 kDa doublet and 51 kDa, the doublet disappeared whilst the band at 51 kDa remained. This showed that the band at 51 kDa was stable to and released by octylglucoside treatment.

**Solubility in Octylglucoside**

Using samples from Group F patients, separation of antigens from elementary bodies using 1-D gel electrophoresis and SDS gave a different staining pattern compared to using 1-D gel electrophoresis and octylglucoside. The 51 kDa band was still visible after octylglucoside. The pair of antigenic bands at 60/62 kDa was not visible in octylglucoside. Therefore a distinguishing character of the 51 kDa antigen of the present invention is its solubility in octylglucoside.

**N-Terminal Amino Acid Sequencing**

N-Terminal amino-acid sequencing was performed upon the 51 kDa band. The resulting sequence was then used to query the Chlamydia Genome Project database which identified the protein of SEQ ID NO: 2 and a *C. trachomatis* homologue.

**Epitope Mapping**

A series of overlapping peptides of 15 amino acids covering the derived amino acid sequence of the protein were synthesised on polyethylene pins with reagents from an epitope scanning kit (Cambridge Research Biochemicals, Cambridge, UK) as described previously by Geysen *et al.* (1987, Journal of Immunological Methods, 102: 259-274). Peptide 1 consisted of residues 1 to 15, peptide 2 consisted of residues 2 to 16 etc. The reactivity of each peptide with patient sera (diluted 1:200) was determined for IgG by ELISA. Data were expressed as A405 after 30 minutes of incubation.

Sera from patients as follows:

Group 1: Children with respiratory tract infection and no evidence of *Chlamydia pneumoniae* as shown by negative immunoblot and microimmunofluorescence (less than 1 in 64) (n = 3).

Group 2: Children with respiratory tract infection, positive immunoblot and microimmunofluorescence test greater than 1 in 512 (n = 6).

Group 3: Patients undergoing cardiac surgery for advanced coronary disease and antibody on immunoblot (n =2).

Group 4: Patients presenting with history of chest pain, negative troponin (<0.2), negative immunoblot (n = 3).

Group 5: Patients presenting with early coronary, positive troponin (>0.2) and antibody on immunoblot (n =8).

Results

*Epitope mapping*

Epitope mapping defined eleven areas where children with acute chlamydial infection produced wells with a mean optical density (OD) greater than 1. In the case of epitopes having SEQ ID NOs: 4, 5, 6, 7, 8, 10, 12 and 14 the mean OD was at least 2 standard deviations above that of Group 1 (children with no evidence of *C.pneumoniae* infections). This applied also to Groups 3, 4 and 5 with the exception of SEQ ID NO: 5 which was positive in Groups 4 and 5.

Peptide 1(SEQ ID NO: 15) representing epitope having the sequence of (i.e. which is carried by the peptides having the sequence of) SEQ ID NO: 8 and peptide 2 (SEQ ID NO: 16) representing the carboxy end of SEQ ID NO: 4, the epitope having the sequence of SEQ ID NO: 5 and the amino end of SEQ ID NO: 6 were synthesised.

Preparation of rabbit polyclonal serum

New Zealand white rabbits were pre-bled and then immunised subcutaneously with either peptide 1 or peptide 2 (0.1 ml of 1 mg/ml) conjugated to KLH suspended in either Freund's adjuvant (injection at day 0) or Freund's incomplete adjuvant on days 14, 42, and 70). Serum was obtained for indirect ELISA at the terminal bleed-out.

Indirect ELISA

By a simple adsorption of each peptide to a microtitre plate the following procedure was performed The peptide was dissolved in 2 ml of 0.01 M phosphate buffer saline (PBS), pH 7.2 and diluted to a concentration of 10 µg/ml (1/100) in the same buffer.

1. 150 µl aliquots of peptide (10 µg/ml in 0.01 M PBS) were pipetted into the wells of a Falcon 3912 microassay plate and were incubated overnight at 4 °C.
2. The unbound peptide was removed by washing four times (4 x 10 minutes) with 0.05% Tween 20 in 0.01 M PBS (pH 7.2).
3. The plates were blocked with 2% skimmed milk-10% FCS in 0.01 M PBS for 1 hour at 37 °C.
4. The plates were washed four times (4 x 10 minutes) with 0. 05% Tween 20 in 0.01 M PBS and the serum under investigation was added (1/100 dilution in blocking solution) into the wells of micro assay plate (three wells used for each serum) and incubated for 2 hours at 37 °C.
5. The plates were washed four times (4 x 10 minutes) with 0.05% Tween 20 in 0.01 M PBS and secondary antibody, anti-rabbit IgG peroxidase conjugate (1/1000 dilution in blocking solution) was added and incubation proceeded for 1 hour at 37 °C.
6. The plates were washed four times (4 x 10 minutes) with 0.05% Tween 20 in 0.01 M PBS, followed by a further washing with 0.01 M PBS. The plate was then incubated for 45 minutes at room temperature with agitation in 0.5

mg/ml of freshly prepared 2,2 Azino-bis [3-ethylbenz-thiazoline-6-sulfonic acid] diammonium (ABTS tablets) in pH 4.0 citrate buffer with 0.01% (w/v) hydrogen peroxide.

7. Optical density (OD) measurements were made with an ELISA plate reader (Titertek Miltiscan) at a wavelength of 405 nm.

8. The average readings for each three wells for each serum was determined.

Results

The results shown in Table 3 demonstrate seroconversion to each individual peptide.

Expression of the amino-end of the protein

The sequence was codon optimised (Genosys, California) for *E.coli* and a BamHI and Not1 site added to opposite ends. The optimised sequence and PET 29 vector (Novagen, Wisconsin) were restriction digested using BamHI and Not1 and transformed by heat shock into *E.coli* strain BL21 (Invitrogen, Carlsbad, California). The expressed amino acids were from amino acids 1-292 and included the epitopes represented by peptides 1 and 2. This construct included an S-tag and Thrombin cleavage site at the amino end and histidine tag at the carboxy end (SEQ ID NO: 3).

Purification

The transformants were expressed as follows. Briefly, 5 ml of an overnight culture was used to inoculate 500 ml LB (50 µg/ml kanamycin, 34 µg/ml chloramphenicol) which was grown for 2 hours at 37 °C to an OD 600 of 0.5, then induced for 3 hours with 0.1 mM IPTG (Sigma, Poole Dorset). The cells were pelleted and disrupted by crushing at -20 °C in an XPRESS. The buffer (50 mm $NaH_2PO_4$, 0.5 M NaCl, 10 mm imidazole) and the cell debris pelleted down. The supernatant was filter sterilised and put on a Ni-NTA agarose slurry affinity column (Qiagen) in order to capture the His-tagged recombinant protein. The column was washed 3 times with 4 ml of washing buffer and

the protein eluted maximally with 150 mM imidazole. The protein gave a single band on a 10% acrylamide gel stained with Coomassie Brilliant Blue with an apparent molecular weight of 37 kDa. On Western blot counterstaining with the anti-His mouse alkaline phosphate conjugate (1:2,500) (Sigma, Dorset, Poole) this produced a single band at 37 kDa and a breakdown product at 35 kDa. The protein concentration of the elute was measured and standardised to 10 mg/ml.

Amino acid sequencing

The protein was amino end cleared to remove the S-tag using a Thrombin cleavage Kit (Novagen). The digestion reaction was 5 µl 10 x Thrombin cleavage buffer, 0.5 mg purified recombinant protein, 1 µl of 0.01 µg/ml Thrombin which was left at room temperature for 18 hours. The reaction mix was run on a 12% SDS-PAGE gel and transferred onto PVDF membrane (Amersham, Chalfont, UK). This was stained with Coomassie Brilliant Blue and the protein bands destained and excised. Direct amino acid sequencing gave amino acids 28-32 of SEQ ID NO: 3 which matched the amino end (Department of Biochemistry, University of Cambridge).

Human recombinant antibodies

These peptides and the purified recombinant proteins were used to pan the phage display library. The peptide and recombinant protein were used at 10 mg/ml on NunC immunotubes Bst-N1 fingerprints of the PCR-amplified ScFv inserts before panning showed a highly heterogeneous library. After panning against peptide 1, 7 fingerprints were identified of which four were represented by more than one clone (A, B, C, D). These were combined as a pool for a neutralisation assay (pool 1) (below). After panning against peptide 2, clone A was present as well as a new ScFv, E. A and E were combined to produce pool 2. Against the clone recombinant fragment ScFvs E, F and G were present as well as a further ScFv, H. ScFvs E, F, G and H were tested together as pool 3.

Neutralisation assays

Chang cells (50 ml of $10^6$ cells/ml) in maintenance media were grown overnight at 37 °C with 5% $CO_2$. Chang cells (1 ml of $1x10^6$ cells/ml maintenance media) were grown overnight at 37 °C with 5% $CO_2$ in plastic bijoux containing a thin glass circle on which the cells can grow. For recombinant protein or peptide assay (0.1 µl/ml), 100 µl of each sample was incubated with shaking for 1 hour with the cells at 37 °C. For the phage and sera assays, 100 µl of each sample (1:10 rabbit sera or dialysed phage pools 1-3) were incubated with 100 µl elementary bodies (EB) for 1 hour at 37 °C, shaking. After this first incubation, the 100 µl EB or 200 ml of the phage or rabbit sera/EB mix was added to the Chang cells. This was incubated with shaking for 1 hour at 37 °C. The supernatant was removed from every sample and replaced by 1 ml of fresh maintenance media. This was incubated at 37 °C with 5% $CO_2$ for 72 hours.

For both assays, the inclusion bodies were fixed and stained the following way; the cells were washed twice with PBS, then fixed with 100% methylated spirits for 10 minutes and washed twice again with PBS. The glass circles were incubated for 30 minutes with 10 µl of mouse *C.pneumoniae* inclusion bodies monoclonals (Mab) then washed 3 times with PBS and incubated for 30 minutes with 100 µl of fluorescein conjugated anti-mouse IgG. The inclusion bodies were then observed by fluorescence microscopy and three 200X fields counted. EB only samples were used as a positive control for chlamydial infection and dialysed phage supernatant without EB as a negative control.

Results

See Table 4 (Table of Neutralisation Assays).

Conclusion

Pre-incubation with the rabbit antiserum against peptide 2 and peptide 2 itself reduced the infectivity due to *C.pneumoniae*. Incubation with peptide 1 produced a similar reduction . The pools of phages were also active.

Overall this demonstrated the immunogenicity of the antigen, the potential therapeutic effect of peptides representing its key epitopes and both rabbit hyperimmune antiserum and ScFvs against these epitopes.

Table 1

| Apparent Molecular Weight (kDa) | Group B (N=18) | | Group C (N=18) | | Group D (N=27) | | Group E (N=21) | |
|---|---|---|---|---|---|---|---|---|
| | IgM | IgG | IgM | IgG | IgM | IgG | IgM | IgG |
| 180 | 1 | 2 | | 2 | 1 | 6 | | 1 |
| 130 | | 2 | | | 1 | 4 | | |
| 120 | 1 | 5 | | 1 | 1 | 5 | | 1 |
| 98 | | 5 | | 1 | 2 | 5 | | 2 |
| 90 | | 2 | | | | 2 | | |
| 67 | | 2 | 5 | 1 | | | 1 | 1 |
| 60/62* | 8 | 5 | 5 | | 13 | 7 | 2 | 2 |
| 51 | 7 | 11 | 9 | 10 | 2 | 3 | 1 | 2 |
| 47 | 1 | 1 | 1 | | 0 | 0 | 0 | 0 |
| 40 | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 1 |
| 30 | | 4 | 0 | 3 | | 2 | | 2 |

* runs as a doublet within 1 mm of each other

Table 2

| | | Value for [a] | | | | |
|---|---|---|---|---|---|---|
| Well No. | Epitope SEQ ID NO | Group 1 (n = 3) | Group 2 (n = 6) | Group 3 (n = 2) | Group 4 (n = 3) | Group 5 (n = 8) |
| 3 | 9 | 0.538±0.205 | 1.028±0.423 | 0.425±0.036 | 0.416±0.184 | 0.499±0.191 |
| 4 | | 0.599±0.252 | 1.487±0.462 | 0.502±0.036 | 0.407±0.107 | 0.438±0.162 |
| 13 | 10 | 0.462±0.203 | 1.103±0.229 | 0.473±0.026 | 0.421±0.162 | 0.427±0.188 |
| 31 | 11 | 0.491±0.192 | 1.103±0.310 | 0.440±0.004 | 0.407±0.105 | 0.310±0.129 |
| 41 | 12 | 0.547±0.235 | 1.169±0.256 | 0.474±0.024 | 0.393±0.08 | 0.376±0.158 |
| 43 | 13 | 0.598±0.258 | 1.223±0.323 | 0.558±0.015 | 0.423±0.119 | 0.406±0.181 |
| 55 | 4 | 0.547±0.235 | 1.265±0.334 | 0.475±0.02 | 0.373±0.076 | 0.381±0.042 |
| 58 | 5 | 0.611±0.019 | 1.025±0.06 | 0.611±0.019 | 1.127±0.253 | 0.800±1.232 |
| 59 | 6 | 0.494±0.166 | 1.096±0.267 | 0.547±0.009 | 0.546±0.200 | 0.702±0.144 |
| 60 | 7 | 0.489±0.129 | 1.048±0.270 | 0.483±0.064 | 0.388±0.008 | 0.449±0.140 |
| 61 | | 0.530±0.236 | 1.051±0.262 | 0.59±0.089 | 0.446±0.09 | 0.784±0.257 |
| 76 | 8 | 0.485±0.158 | 1.174±0.255 | 0.654±0.068 | 0.564±0.223 | 0.666±0.266 |
| 79 | 14 | 0.510±0.235 | 1.21±0.273 | 0.418±0.003 | 0.423±0.127 | 0.388±0.153 |

[a] Optical density ± Standard deviation

Table 3

| | [a] Pre Serum | Post Serum |
|---|---|---|
| Peptide 1 | 0.055 ± 0.01 | 0.591 ± 0.06 |
| Peptide 2 | 0.056 ± 0.01 | 0.507 ± 0.04 |

[a] optical density ± standard derivation

Table 4 - Table of Neutralisation Assays

| | Number of Elementary Bodies in Three 200x Fields |
|---|---|
| Cell control (dialysed phage supernatant) | 0 |
| Cell control (elementary bodies) | 30 |
| Rabbit anti-serum | |
| Versus peptide 1 | 30 |
| Versus peptide 2 | 19 |
| Pre-incubation | |
| Peptide 1 | 13 |
| Peptide 2 | 0 |
| Recombinant protein | 12 |
| Phage Pools | |
| Pool 1 | 18 |
| Pool 2 | N/D |
| Pool 3 | 21 |

# CLAIMS

1. A *C.pneumoniae* protein having the amino acid sequence of SEQ ID NO: 2 for use in a method of treatment or diagnosis of the human or animal body.

2. A nucleotide sequence encoding a protein according to claim 1 for use in a method of treatment of the human or animal body.

3. A nucleotide sequence according to claim 2, having the sequence of SEQ ID NO: 1.

4. The use of a protein, immunogenic fragment thereof or nucleotide sequence encoding same according to any one of the preceding claims in the manufacture of a medicament for the treatment of infection due to *C.pneumoniae*.

5. The use of an immunogenic fragment according to claim 4, having the amino acid sequence of any one of SEQ ID NOs: 4-14 in the manufacture of a medicament for the treatment of infection due to *C.pneumoniae*.

6. The use of an inhibitor specific against the protein, immunogenic fragment or nucleotide sequence encoding same according to any one of the preceding claims in a method of manufacture of a medicament for the treatment of infection due to *C.pneumoniae*.

7. The use of an inhibitor according to claim 6, the inhibitor being selected from the group of an antibody, DNA vaccine, ribozyme and antisense oligonucleotide.

8. A method of manufacture of a medicament for the treatment of infection by *C.pneumoniae* characterised in the use of a protein, immunogenic fragment thereof or nucleotide sequence encoding same according to either one of claims 4 or 5.

9. A method of manufacture of a medicament for the treatment of infection due to *C.pneumoniae* characterised in the use of an inhibitor according to either one of claims 6 or 7.

10. The use of a protein according to claim 1 or an immunogenic fragment thereof or a binding agent specific to same or an inhibitor of same in the manufacture of a diagnostic test for *C.pneumoniae*.

11. A kit of parts for a diagnostic test for *C.pneumoniae*, characterised in that it comprises a protein according to claim 1 or an immunogenic fragment thereof or a binding agent specific to same or an inhibitor of same.

12. A diagnostic test method for infection due to *C.pneumoniae* comprising the steps of:

i) reacting an antibody specific against the protein according to claim 1 with serum from a patient;

ii) detecting an antibody - antigen binding reaction; and

iii) correlating the detection of an antibody - antigen binding reaction with the presence of the protein.

13. A diagnostic test method according to claim 12, being a method of diagnosis of the human or animal body.

14. A method of treatment of infection due to *C.pneumoniae* comprising the step of administering to a patient a medicament comprising a protein, immunogenic fragment thereof, nucleotide sequence encoding same or an inhibitor thereof according to any one of claims 4-7.

SEQUENCE LISTING

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            420                 425                 430

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465                 470                 475                 480

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Gln Gly Lys Asn Thr Glu Met Glu Ser Asp Ala Thr Ile Ala Gly Ala
    50                  55                  60
Ser Gly Lys Asp Lys Thr Ser Ser Thr Thr Lys Thr Glu Thr Ala Pro
65                  70                  75                  80
Gln Gln Gly Val Ala Ala Gly Lys Glu Ser Ser Glu Ser Gln Lys Ala
                85                  90                  95
Gly Ala Asp Thr Gly Val Ser Gly Ala Ala Ala Thr Thr Ala Ser Asn
            100                 105                 110
Thr Ala Thr Lys Ile Ala Met Gln Thr Ser Ile Glu Glu Ala Ser Lys
        115                 120                 125
Ser Met Glu Ser Thr Leu Glu Ser Leu Gln Ser Leu Ser Ala Ala Gln
    130                 135                 140
Met Lys Glu Val Glu Ala Val Val Val Ala Ala Leu Ser Gly Lys Ser
145                 150                 155                 160
Ser Gly Ser Ala Lys Leu Glu Thr Pro Glu Leu Pro Lys Pro Gly Val
                165                 170                 175
Thr Pro Arg Ser Glu Val Ile Glu Ile Gly Leu Ala Leu Ala Lys Ala
            180                 185                 190
Ile Gln Thr Leu Gly Glu Ala Thr Lys Ser Ala Leu Ser Asn Tyr Ala
        195                 200                 205
Ser Thr Gln Ala Gln Ala Asp Gln Thr Asn Lys Leu Gly Leu Glu Lys
    210                 215                 220
Gln Ala Ile Lys Ile Asp Lys Glu Arg Glu Glu Tyr Gln Glu Met Lys
225                 230                 235                 240
Ala Ala Glu Gln Lys Ser Lys Asp Leu Glu Gly Thr Met Asp Thr Val
                245                 250                 255
Asn Thr Val Met Ile Ala Val Ser Val Ala Ile Thr Val Ile Ser Ile
            260                 265                 270
Val Ala Ala Ile Phe Thr Cys Gly Ala Gly Leu Ala Gly Leu Ala Ala
        275                 280                 285
Gly Ala Ala Val Gly Ala Ala Ala Ala Gly Gly Ala Ala Gly Ala Ala
    290                 295                 300
Ala Ala Thr Thr Val Ala Thr Gln Ile Thr Val Gln Ala Val Val Gln
305                 310                 315                 320
Ala Val Lys Gln Ala Val Ile Thr Ala Val Arg Gln Ala Ile Thr Ala
                325                 330                 335
Ala Ile Lys Ala Ala Val Lys Ser Gly Ile Lys Ala Phe Ile Lys Thr
            340                 345                 350
Leu Val Lys Ala Ile Ala Lys Ala Ile Ser Lys Gly Ile Ser Lys Val
        355                 360                 365
Phe Ala Lys Gly Thr Gln Met Ile Ala Lys Asn Phe Pro Lys Leu Ser
    370                 375                 380
Lys Val Ile Ser Ser Leu Thr Ser Lys Trp Val Thr Val Gly Val Gly
385                 390                 395                 400
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Val Val Val Ala Ala Pro Ala Leu Gly Lys Gly Ile Met Gln Met Gln
                405                 410                 415
Leu Ser Glu Met Gln Gln Asn Val Ala Gln Phe Gln Lys Glu Val Gly
            420                 425                 430
Lys Leu Gln Ala Ala Ala Asp Met Ile Ser Met Phe Thr Gln Phe Trp
        435                 440                 445
Gln Gln Ala Ser Lys Ile Ala Ser Lys Gln Thr Gly Glu Ser Asn Glu
    450                 455                 460
Met Thr Gln Lys Ala Thr Lys Leu Gly Ala Gln Ile Leu Lys Ala Tyr
465                 470                 475                 480
Ala Ala Ile Ser Gly Ala Ile Ala Gly Ala His Lys Thr Asn Asn Phe
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<213> Artificial Sequence

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<222> (1)..(30)
<223> S-tag and thrombin cleavage site

<220>
<221> UNSURE
<222> (292)..(302)
<223> Histidine tag

<400> 3

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Pro Asp Leu Gly Thr Leu Val Pro Arg Gly Ser Ala Ile Ser Asp Pro
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Asp Thr Asn Met Ser Ile Ser Ser Ser Ser Gly Pro Asp Asn Gln Lys
        35                  40                  45

Asn Ile Met Ser Gln Val Leu Thr Ser Thr Pro Gln Gly Val Pro Gln
    50                  55                  60

Gln Asp Lys Leu Ser Gly Asn Glu Thr Lys Gln Ile Gln Gln Thr Arg
65                  70                  75                  80

Gln Gly Lys Asn Thr Glu Met Glu Ser Asp Ala Thr Ile Ala Gly Ala
                85                  90                  95
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Ser Gly Lys Asp Lys Thr Ser Ser Thr Thr Lys Thr Glu Thr Ala Pro
100 105 110

Gln Gln Gly Val Ala Ala Gly Lys Glu Ser Ser Glu Ser Gln Lys Ala
115 120 125

Gly Ala Asp Thr Gly Val Ser Gly Ala Ala Ala Thr Thr Ala Ser Asn
130 135 140

Thr Ala Thr Lys Ile Ala Met Gln Thr Ser Ile Glu Glu Ala Ser Lys
145 150 155 160

Ser Met Glu Ser Thr Leu Glu Ser Leu Gln Ser Leu Ser Ala Ala Gln
165 170 175

Met Lys Glu Val Glu Ala Val Val Val Ala Ala Leu Ser Gly Lys Ser
180 185 190

Ser Gly Ser Ala Lys Leu Glu Thr Pro Glu Leu Pro Lys Pro Gly Val
195 200 205

Thr Pro Arg Ser Glu Val Ile Glu Ile Gly Leu Ala Leu Ala Lys Ala
210 215 220

Ile Gln Thr Leu Gly Glu Ala Thr Lys Ser Ala Leu Ser Asn Tyr Ala
225 230 235 240

Ser Thr Gln Ala Gln Ala Asp Gln Thr Asn Lys Leu Gly Leu Glu Lys
245 250 255

Gln Ala Ile Lys Ile Asp Lys Glu Arg Glu Glu Tyr Gln Glu Met Lys
260 265 270

Ala Ala Glu Gln Lys Ser Lys Asp Leu Glu Gly Thr Met Asp Thr Val
275 280 285

Asn Thr Val Ala Ala Ala Leu Glu His His His His His His
290 295 300

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<212> PRT
<213> Chlamydia pneumoniae

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<210> 5
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<213> Chlamydia pneumoniae

<400> 5
Pro Lys Pro Gly Val Thr Pro
1 5

<210> 6
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Gly Val Thr Pro Arg Ser Glu Val Ile
1 5

<210> 7
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<210> 8
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Ala Ile Lys Ile Asp Lys Glu Arg
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<210> 9
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1 5

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<400> 10

Ser Gly Asn Glu Thr Lys Gln Ile Gln
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<210> 11
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<212> PRT
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1 5

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1 5

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1 5

<210> 14
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Glu Tyr Gln Glu Met Lys Ala Ala Glu
1 5

<210> 15
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<212> PRT
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Glu Lys Gln Ala Ile Lys Ile Asp Lys Glu Arg Glu Glu Tyr
1 5 10

<210> 16
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<212> PRT
<213> Chlamydia pneumoniae

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1 5 10